PATENT COOPERATION TREATY

De Clercq, Brants & Partners cv - RECEIVED - 22 APR. 2005 DDC/NB-VP

9 2345

by fax and post

PCT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DE CLERCQ, Ann
De Clercq, Brants & Partners
E. Gevaertdreef 10a
B-9830 Sint-Martens-Latem
BELGIQUE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	20.04.2005

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
PAM-014-PCT	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/13601	02.12.2003	04.12.2002

Applicant
PAMGENE BV et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Cleere, C Tel. +49 89 2399-7713

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PAM-014-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/13601	International filing date *(day/month/year)* 02.12.2003	Priority date *(day/month/year)* 04.12.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant PAMGENE BV et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.07.2004	20.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Madlener, M Telephone No. +49 89 2399-7705

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/13601

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-47 as originally filed

Claims, Numbers

1-35 received on 04.11.2004 with letter of 04.11.2004

Drawings, Sheets

1/8-8/8 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30, 34-35
	No:	Claims	31-33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-35
Industrial applicability (IA)	Yes:	Claims	16-35 (no opinion with regard to claims 1-15)
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 1 in its current wording would not appear to exclude surgical steps practiced on the human or animal body and is hence considered to relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US 5,741,647 (April 21, 1998)
D2: US 6,020,187 (February 1, 2000)
D3: WO 96/15271 (May 23, 1996)
D4: US 5,843,767 (December 1, 1998)

It appears that the amendments to the claims do not extend the subject-matter of the present application beyond the content of the application as originally filed.

NOVELTY:

1. Claim 31 relating to a "device for flow-through hybridization .." has to be construed as "device suitable for flow-through hybridization.." (Guidelines III-4.8).

   In response to the previously raised objections (in view of **D4**), applicant has argued essentially that (i) D4 does not disclose devices comprising a fibre network matrix permitting immobilization of intact genomic DNA and that (ii) D4 does not disclose or suggest the immobilization of intact genomic contents. With regard to (ii), it is noted that claim 31 has to be construed as explained above. With regard to (i) it is noted that, independently of D4, any **standard device like, e.g., a 96-well plate**

comprising a cellulose or nylon filter would appear to anticipate the device according to **claims 31-32** pursuant to Article 33(2) PCT.

Since the use of such standard devices for hybridization purposes in methods involving suction during washing steps is likewise considered to have been commonplace, **claim 33** is also considered to lack novelty over such standard devices pursuant to Article 33(2) PCT.

INVENTIVE STEP:

1.a In response to the previous objections raised in view of **D1 or D2** alone or in combination with the skilled person's common general knowledge, applicant has argued essentially that (i) the genomic DNA samples referred to in D1 and D2 "are not related to intact genomic DNA" and that (ii) the choice of a "somewhat larger pore size" would not appear suitable to establish inventive step, concluding that "neither D1 nor D2 disclose or suggest the detection of gene copies in an intact chromosomal DNA or RNA sample which is immobilized within a matrix assuring the most favourable hybridisation kinetics (see present application, p.3, l.22-28) and therefore D1 and D2 do not anticipate or render obvious the presently claimed subject-matter."

1.b This argumentation has been carefully considered. However, the authority entrusted with preliminary examination cannot agree to it, the reasons being as follows (in the following, passages in D1 are cited for the sake of simplicity; identical disclosure can also be found in D2):

D1 discloses a method for hybridizing probes onto immobilized genomic DNA comprising essentially steps (a)-(d) of claim 1 of the present application (cf., e.g., D1, abstract; col. 2, ll. 20-3; col. 5, l. 62 - col. 6, l. 59; Example I-III). The method of **amended claim 1** differs from D1 in that it requires the immobilization of undigested or intact, denatured genomic DNA within a matrix comprising pore sizes within a range of 0.6 µm to 2 µm including the outer limits. Thus, the underlying problem may be formulated as the provision of a modified hybridisation method which is suitable and provides the advantages disclosed in D1 (reduced hybridization time; reduced amounts of reagents; cf. D1, col. 2, ll. 20-24) also for undigested or intact, denatured genomic DNA. The method of amended claim 1 provides a solution. However, this

solution can for the following reasons not be considered inventive in the sense of Article 33(3) PCT.

D1 teaches that its invention "can provide the controlled conditions critical for the hybridisation process which can be universally applied to all cases [/] that is applicable to conventional Southern, Northern, Dot-Blot, Slot-Blot and Reversed-Dot Blot hybridisation techniques so far reported in the literature" (D1, col. 3, ll. 41-46) and that it "will work the same on various lengths of the target nucleic acid sequences" (D1, col. 5, ll. 10-12).

Given the fact that it was commonly known in the art to use also undigested genomic DNA for Southern Blotting, the skilled person seeking to solve the above-formulated could and would have applied the method of D1 for this purpose. In doing so, he/she would have chosen other pore sizes, namely larger ones, in order to adapt to the larger size of the molecules to be immobilized. In fact, D1 explicitly discloses that "a large part of the DNA will be immobilized in the interior part of the membrane" (D1, col. 2, ll. 64-65) and that flow through hybridisation processes eliminate disadvantages associated therewith in conventional, i.e., non-flow through hybridization methods (D1, col. 3, ll. 1-7) and in addition yields the above-mentioned advantages reduced hybridization time; reduced amounts of reagents; cf. D1, col. 2, ll. 20-24, cf. also D1, col. 4, ll. 4-23).

Thus, **amended claims 1-2** are considered to violate Article 33(3) PCT.

1.c **D1** also discloses the additional features of dependent **claims 4** (cf., e.g., D1, Ex. I), **5-10** (cf., e.g., D1, col. 6, ll. 21-24;), **11** (cf., e.g., D1, Ex. I-II), **12** (cf., e.g., D1, col. 6, ll. 14-18), which are hence also considered to lack the inventive step required by Article 33(3) PCT

1.d Analogous arguments apply to **claims 16-17** (cf., e.g., D1, Ex. I-III; claim 6), which are hence also considered to lack the inventive step required by Art. 33(3) PCT.

1.e In view of the fact that the concept of common primer binding sequences, allowing for the amplification of a plurality of sequences using a single set of common primers, and a single set of amplification conditions optimized for the common primers, was

known in the art (cf., e.g., D3, abstract; p. 15, l. 9-15), the methods according to **claim 15 or claim 18** as well as the kit according to **claim 35** are likewise considered to lack the inventive step required by Article 33(3) PCT in view of **D1 or D2 in combination with the skilled person's common general knowledge reflected by, e.g., D3**.

Applicant has counterargued essentially that inventive step of claim 15 of claim 18 follows from the fact that the method of amended claim 1 is inventive. Since this is not agreed to, applicant's further line of argumentation cannot be agreed either.

1.f Given the fact that the concept of wells exposing a matrix (cf., e.g., essentially any 96-well-plate) was known in the art prior to the effective date and is hence considered novelty-destroying to **claims 31-33** (cf. *supra*) and that mere packaging of anticipating subject-matter in a kit including instructions, the subject-matter of **claims 34-35** is considered obvious pursuant to Article 33(3) PCT.

1.g The additional features of **claims 2-3, 9-10, 13-14, 19-30 and 32** would likewise not appear suitable to establish the inventive step required by Article 33(3) PCT, the reason being that they would appear to represent standard, i.e., non-inventive variations in the pertinent art.

2. The same objections arise with regard to **D2**, the description of which is essentially identical to that of D1 (cf., e.g., D2, abstract; Fig. 1-4; col. 2, ll. 22-37; col. 3, l. 54 - col. 4, l. 31; col. 6, ll. 29-32; Ex. I-III).

Claims (retyped)

1. Method for hybridization of probes onto immobilized genomic DNA comprising the steps of:
   (a) providing a sample containing or suspected of having genomic content, wherein said genomic content is undigested or intact chromosomal DNA or RNA,
   (b) denaturing said intact genomic content;
   (c) immobilizing said denatured intact genomic content within a matrix; said matrix comprising pore sizes within a range of 0.6 µm to 2 µm including the outer limits;
   (d) providing a set of probes and passing said probes through said matrix under conditions favouring hybridization of the probes to its complementary sequence in said intact genomic content; and
   (e) washing off non-hybridized probes through said matrix, leaving formed hybridized intact genomic content/probe complexes for further analysis.

2. Method according to claim 1, wherein said denatured intact genomic DNA is permeated within said matrix.

3. Method according to any of claims 1 or 2, wherein said probes are passed through said matrix by at least one cycle of alternating downwards and upwards flow.

4. Method according to any of claims 1 to 3, wherein said washing step is carried out by passing through said matrix a wash fluid by at least one cycle of downwards flow.

5. Method according to any of claims 1 to 4, wherein said matrix is a membrane.

6. Method according to claim 5, wherein said membrane comprises a 3D network structure.

7. Method according to claim 6, wherein said network structure is a flow-through structure.

8. Method according to claim 6 or 7, wherein said network structure is a fibre network structure.

9. Method according to claim 8, wherein said fibre is of vegetable origin.

10. Method according to claim 9, wherein said fibre is cellulose.

11. Method according to any of claims 1 to 10, wherein the matrix allows for a flow rate comprised between 50mm/30min and 250mm/30min including the outer limits.

12. Method according to any of claims 1 to 11, wherein said matrix is activated with an affinity conjugate.

13. Method according to claim 12, wherein said affinity conjugate is chosen from the group comprising poly-L-lysine, poly-D-lysine, 3-aminopropyl-triethoxysilane, poly-arginine, polyethyleneimine, polyvinylamine, polyallylamine, tetraethylenepentamine, ethylenediamine, diethylenetriamine, triethylenetetramine, pentaethylenehexamine and hexamethylenediamine.

14. Method according to claim 13, wherein said affinity conjugate is poly-L-lysine.

15. Method according to any of claims 1 to 14, wherein said probes are flanked by primer binding sequences.

16. Use of a method according to any of claims 1 to 15 for intact genomic DNA hybridisation.

17. Use of a method according to any of claims 1 to 15 for detection and quantification of target nucleic acids in an intact genomic DNA sample.

18. Method for target nucleic acid detection and quantification in an intact genomic DNA sample comprising the steps of:
(a) providing intact genomic DNA and denaturing said intact genomic DNA;
(b) performing a hybridisation according to a method as described in any of claims 1 to 15;
(c) recovering hybridised probes; and essentially simultaneously amplifying any recovered probe using a single primer pair, each member of said primer pair binding to each recovered probe onto the respective flanking primer binding sequences of said probe; and,
(d) qualitatively and quantitatively analysing the recovered amplified probes of step (c).

19. Method according to claim 18, wherein the analysis of step (d) is by microarray analysis.

20. Method according to claim 18 or 19, wherein each probe is flanked 5' and 3' by primer binding regions with said 5' and 3' flanking primer binding sequences being the same or substantially the same for each probe.

21. Method according to any of claims 18 to 20, wherein said amplification of step (c) is a quantitative amplification.

22. Method according to claim 21, wherein said amplification is by means of polymerase chain reaction.

23. Method according to any of claims 18 to 22, wherein the amplified probes are provided with a label.

24. Method according to claim 23, wherein said label is a fluorescent label.

25. Use of a method according to any of claims 18 to 24 for genomic screening.

26. Use of a method according to any of claims 18 to 24 for detecting deletions or duplications in genomic DNA.

27. Use of a method according to any of claims 18 to 24 for genome profiling.

28. Use of a method according to any of claims 18 to 24 for identifying and quantitatively detecting the degree of pathogenesis, disease or contamination in a sample.

29. Use of a method according to any of claims 18 to 24, for identifying and detecting the presence of infectious agents in a sample.

30. Use of a method according to any of claims 18 to 24, for genotyping pathogens present in a sample.

31. Device for flow-through hybridisation of probes onto immobilized intact genomic DNA comprising a well holder, said well holder comprising one or more round wells with a fixed diameter, said wells exposing a fibre network matrix, said matrix comprising pore sizes within a range of 0.6 µm to 2 µm including the outer limits; wherein said matrix permits immobilization of intact genomic DNA and which allows hybridisation of said immobilized intact genomic material with probes by flow-through hybridisation.

32. Device according to claim 31, wherein said matrix permits permeation of intact genomic DNA.

33. Apparatus for flow-through hybridisation of probes onto immobilized genomic DNA comprising:
    (a) a device according to claim 31 or 32;
    (b) means for addition of a controlled amount of fluid to at least one of the wells of the device as described in (a);
    (c) means for applying and/or maintaining a controlled pressure difference over the matrix in each of the wells.

34. Kit for flow-through hybridisation of probes onto immobilized intact genomic DNA comprising :
    (a) a device according to claim 31 or 32; and

(b) instructions to carry out a method according to any of claims 1 to 15 or 18 to 24.

35. Kit according to claim 34, additionally comprising:

(a) a set of probes, wherein each probe is flanked 5' and 3' by primer binding regions with said 5' and 3' flanking primer binding sequences being the same or substantially the same for each probe;

(b) a single primer pair, each member of said pair being complementary to a primer binding region;

(c) optionally amplification components allowing the amplification of any recovered hybridised probe; and

(d) optionally a microarray, said microarray allowing analysis of the hybridisation results obtained by a method according to any of claims 1 to 15 and 18 to 24.